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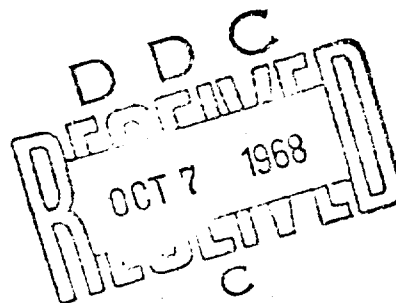
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## METHOD OF ACTION OF BACTERIAL ENDOTOXINS

### II. Circulatory Troubles in Animals Intoxicated by an Endotoxin

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#### INTRODUCTION

The circulatory troubles which appear in animals treated with strong doses of bacterial endotoxins are of such an importance that we think it necessary to devote a systematic study to them. Several personal observations which will be reported here have already been the subject of preliminary communications. Others are entirely original. Lastly, some are not directly the product of our research but were found by us in foreign publications.

#### SYSTEMATIC STUDY OF CIRCULATORY TROUBLES IN ANIMALS INTOXICATED BY A BACTERIAL ENDOTOXIN

1. Examination of the Heart. -- We carried out this work on the guinea pig. As the intoxication progressed, the cardiac beats accelerate more and more; at the same time they lose their normal strength. However, they remain regular until the terminal phase of the intoxication. The stopping of the heart can be progressive. At times it is abrupt and follows, for example, an inopportune examination of the animal. A polypnea generally accompanies the tachycardia.

2. Examination of the Arterial Pressure. -- The study of the arterial pressure (measured at the carotid), in rabbits intoxicated by lethal doses of endotoxins, enabled our colleague and friend, Dr. Paul Boquet, to obtain the following results (still unpublished): the arterial pressure maintains

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itself quite a lengthy period at normal figures; it then collapses.

3. Study of the Peripheral Circulation. -- Hardly two hours after the injection of a strong dose of endotoxin (for example, typhoid), the animals no longer bleed, and it then becomes almost impossible to take a drop of blood, either at the ear (guinea pig) or at the tail (mouse). In addition, the following experiments showed us, in a precise manner, deep-seated troubles in the peripheral circulation [1].

Guinea pigs intoxicated by a strong dose of typhoid endotoxin administered intraperitoneally and normal guinea pigs receive at the same time (1) intracutaneously (abdomen): 1/2 cc of sterile bouillon; (2) in the vein, 4/10 cc of a solution of 1% blue trypan.

One-half hour later, we noted in the normal animals an accumulation of the trypan blue (deep blue color) in the cutaneous area which received the bouillon. On the contrary, in the intoxicated guinea pigs the accumulation of the trypan blue is very slightly evident, and the sicker the animal, the less it is evident. During the hours which follow, the entire tegument of the normal guinea pigs slowly assumes a blue color whereas the skin of the intoxicated guinea pigs remains pale.

We were able to reproduce this experiment recently, with identical results, in the rabbit. This delay in the normal diffusion of the blue trypan and in its accumulation at the inflammatory centers indicates very well the importance of the circulatory troubles in intoxicated animals (at least in those which appear in the skin).

4. Study of the Capillary Vessels. -- This furnished us with particularly instructive results.

a) We can note, first of all, in the animals subjected to the action of a bacterial endotoxin, an extremely sharp increase in the strength of the capillaries [1]. In the guinea pig, we measured this strength by the classical method by determining, with a water suction pump the smallest depression which is sufficient to break the vessels when it is maintained at a given point (for instance, in the dorsal cutaneous area) during one minute. The results are expressed in centimeters of Hg. Our experimental animals were divided into two lots. Those of the first lot received 1 cc of saline solution in the peritoneum, and those of the second received variable doses of typhoid endotoxin in the same area. At 30 minutes, one hour and four hours after the injections, the capillary strength of all the guinea pigs was measured again.

Under these conditions we obtained the following results: the capillary strength of the saline-treated animals was not modified or increased slightly at the end of one hour; for example, it went from 40 to 50 cm of Hg. Furthermore, this increase was always only transitory. On the other hand, the capillary strength of the endotoxin-treated guinea pigs rose in considerable proportions, as shown by the few figures obtained within the space of one hour: 15 → > 65, 20 → > 65, 10 → > 60, 25 → > 60 cm of Hg, etc. All of our results, without a single exception, were in agreement. We clearly observe this increase in strength at the end of 30 minutes, in guinea pigs which received only a fraction of a milligram of poison and whose general condition is still satisfactory. In any case, it is durable and is maintained at least several hours.

This large increase of capillary strength in animals treated by a bacterial endotoxin, such as we observe by the method used, must certainly be due to several factors. It is at least certain that it is not entirely secondary to a direct action of the endotoxin on the wall of the capillaries. In fact, in the serum of animals intoxicated by typhoid, we were able to demonstrate the presence [2] of substances different from endotoxin and capable themselves of raising the strength of the capillary vessels of normal guinea pigs very sharply. In all probability, these substances are liberated by the organism itself during the intoxication and are of a hormonal nature. Later we shall have the opportunity of studying them carefully.

b) Another phenomenon in animals intoxicated by an endotoxin which coincides with the increase of the vascular strength, also indicates a profound modification of the circulatory system. It manifests itself by an inhibition of diapedesis. We discovered it in 1941 and since then we have actively pursued the examination [3]. Let us recall its characteristics. In animals (guinea pigs, rabbits, rats, mice, etc.) treated with lethal or sublethal doses of endotoxin, diapedesis is no longer produced. The inflammatory reactions which develop in them are not infiltrated by polynucleates of sanguineous origin, as we see in the normal condition. We can easily imagine the consequences of such a disturbance: the microbes which gave birth to these inflammatory reactions, spared by those powerful white corpuscle phagocytes, can multiply without restraint and possibly swarm through the entire organism. This suppression of polynucleate diapedesis lasts as long as the acute phase of the intoxication. It continues until death, if lethal doses of endotoxin were used.

When we discovered this phenomenon in 1941, we believed that it was characteristic of intoxication by an endotoxin.

For several reasons we had to revise this opinion. First of all, because we ourselves found an inhibition of diapedesis during certain phenomena of shock: anaphylactoid shocks [4], traumatic and toxic shocks [5]. Furthermore, various American authors have observed this, in research completely independent from ours; R. H. Rigdon [6] and Cl. C. Lushbaugh [7] have noted its existence in animals intoxicated by ethyl alcohol. Lastly, systematic bibliographic research enabled us to discover that the same phenomenon had again been noted, as of 1939, but only incidentally and without the authors granting it great importance; for example, A. Penner and A. I. Bernheim pointed out in that year the absence of leucocytal infiltration in the necrotic centers discovered in the autopsy of subjects who died in a state of operational shock[8].

At the present time, we are investigating the mechanism of this inhibition of diapedesis. We already know that it is impossible to implicate either some alteration of the polynucleates under the action of the endotoxins (since these poisons are not leucocidins as we have shown in our previous papers [9]), or a repulsion of the white corpuscles by these same products as was asserted by H. R. Morgan and H. C. Upham [10]. It is more probable that this phenomenon is the consequence of large disturbances occurring in the circulatory current of the intoxicated animals, disturbances which themselves probably result from the change in size of the various vessels: arterioles, capillaries and veinlets. We shall also return to this point later.

c) Another remark should finally be made on the subject of capillary circulation. In animals treated with a bacterial endotoxin, we do not observe, at the same time as diapedesis inhibition, a suppression of the "plasmatexodie." The filtration of the plasma, through the capillary walls, continues in an appropriate manner in the intoxicated subjects. We were able to verify this ourselves.

5. Modifications in the Blood Content of Figured Elements and of Glucose. -- In addition, we observe in animals subjected to the action of strong doses of bacterial endotoxin important modifications in the constitution of the blood itself.

a) Thus, the normal number of the figured elements is markedly disturbed.

In the first place, we note a leucopenia which is usually extremely clear. This leucopenia (which concerns both the polynucleates and the mononucleates) has been pointed out by numerous authors [11]. We also found it consistently [12].

It continues, while becoming more and more marked, until the death of the animals, or in those animals which survive, it finally gives way to a more or less high leucocytosis. In general, this leucopenia appears less than one hour after the injection of the endotoxin. In the surviving animals, it has always disappeared at the end of 24 hours. Its cause seems to be a redistribution of the leucocytes in the vascular system.

At the same time as this leucopenia, we usually observe a slight increase in the number of red corpuscles. Two American authors, A. Penner and A. I. Bernheim, have studied at length the hemoconcentration which appears in animals treated with extracts of Shiga bacillus [14]. They measured it according to the hemoglobin concentration of the blood. In their opinion, such hemoconcentration results from a loss of plasma, but they could not indicate the whereabouts of the lost plasma, i.e., whether it left the blood stream to infiltrate cells and tissue, or whether it is retained in certain deep capillaries the circulation of which is unduly slowed down. In any case, according to Penner and Bernheim, the specific density of the plasma in strongly intoxicated animals does not undergo any change.

b) Lastly, there is a final type of blood disturbances which we shall emphasize in terminating this enumeration; it is the question of troubles due to glycemia. Various authors [14, 15] were impressed by the presence, in experimental animals, at the beginning of bacterial endotoxin intoxication, of a strong hyperglycemia accompanied by a hyperlactacidemia, and they all admitted that this hyperglycemia probably indicated a hypersecretion of adrenaline by the still-intact suprarenals. When the intoxication is prolonged, this hyperglycemia would be succeeded by a hypoglycemia which would be revealed during autopsy by large suprarenal lesions and by the disappearance of the "chromaffin" substance observed by Dennis [16].

## DISCUSSION

I. -- Firstly, let us summarize the diverse information that we have just provided and which indicates that significant circulatory troubles take place in animals intoxicated by a bacterial endotoxin. These troubles are the following:

Clear terminal hypotension, following an arterial pressure which was maintained normal for a long time.

Marked troubles of the peripheral circulation.

Capillary modifications which result in (a) a considerable increase of the strength of the capillary walls, (b)

maintenance of the permeability of these walls to dissolved substances, (c) suppression of all white cell diapedesis. Leucopenia that is always very great (by cellular redistribution) and hemoconcentration.

Troubles with glycemc regulation: hyperglycemia (with hyperlactacidemia) then hypoglycemia.

II. -- This enumeration seems instructive to us inasmuch as it permits an immediate comparison of the state of intoxication produced by a bacterial endotoxin with another pathological process, known as traumatic shock. In traumatic shock, as defined by classical data, we find most of the symptoms that we have just noted. In a shock patient, the arterial pressure remains normal for a certain time before it collapses, and furthermore, the peripheral circulation is extremely disturbed. At the present time, we even have a tendency of making such a phenomenon the true substratum of the state of shock, so much that, for W. W. Swingle, the characteristic sign of shock would be the cessation of all hemorrhage in the patient. Also, in traumatic shock we observe continuation of the permeability of the capillary walls to plasma. This permeability would even be increased, and certain authors have not hesitated to make an exaggerated "plasmatexodie" one of the essential determinant causes of the states of shock. In addition, we ourselves have shown that leucocyte diapedesis was eliminated in shock patients, and we know that we often find hyperglycemia and hyperlactacidemia (with production of a state of acidosis) in these patients. On the other hand, what we find less frequently in states of traumatic shock is a leucopenia (it seems that there is usually a leucocytosis). However, this leucopenia occurs in certain processes which, although somewhat different from traumatic shock, nevertheless represent shock as, for example, certain anaphylactic reactions. Lastly, we shall add that, from the anatomical-pathological standpoint, the lesions that we discover in shock patients (oedematous, congestive, hemorrhagic, necrotic, etc., lesions) are exactly those that we find in subjects intoxicated by an endotoxin, and that when a cure occurs in all these subjects, it occurs under the same conditions -- suddenly.

For all these reasons, we think we have the right, after A. Penner and A. I. Bernheim [14], of equating the state of intoxication produced by a bacterial endotoxin to a state of traumatic shock.

In our opinion, a bacterial endotoxin injected into a sensitive animal does not exercise its ravages by a direct action on the tissues: it merely starts a simple state of shock.



For us, the bacterial endotoxins should, therefore, be classified among the known factors capable of producing shock.

III. -- Undoubtedly, to equate the state of intoxication produced by an endotoxin to a state of shock is not sufficient to resolve the entire problem of the method of action of this bacterial poison "in vivo." However, as we are now going to demonstrate, this identification presents a great interest for several reasons.

a) First of all, it permits understanding why the same weights of endotoxins all cause the same clinical syndrome in the animal, regardless of the germ which furnished them and, hence, regardless of their specificity. Such a fact was very surprising at the time of the isolation (relatively recent) of these endotoxins. Because of the concept that we are contributing today concerning the method of action, "in vivo," of these poisons, we think that it is no longer surprising. In fact, if such endotoxins as typhoidal, colibacillary, choleraic, etc., are toxic, it is not because they possess a "primary" toxicity but simply because they are all shock factors.

b) The fact that microbial toxins are capable, in themselves, of producing clinically characteristic shock phenomena also reinforces the theory that started just after the other war, which places shock under the subordination of hypothetical toxic factors. But let us remember very well, the toxic factor here plays only a trigger role; we should not use its toxicity to explain the gravity of the shock. To us, the gravity of the latter appears to depend only on the violence of the reaction of the organism.

Furthermore, we may wonder whether the endotoxins are the only microbial products capable of behaving like shock factors. In the present status of our research, it is still quite difficult to reply. All that we can state is that, in our hands, only the endotoxins, after subcutaneous injection, resulted in typical states of shock. The extracts (autolysates) of numerous Gram-positive bacteria, the various components of Gram-negative bacteria other than the glucide-lipoid complexes, introduced into the organism in the same way, sometime gave marked local disorders but were not accompanied by a general intense reaction of the organism. Undoubtedly, we should make an exception for the exotoxins (diphtheric, tetanic, staphylococcic, etc.), but the clinical table that we obtain in this case is very different from that observed in animals treated by an endotoxin.

However, we may question whether the sudden injection into the blood of microbial extracts, which are not very

harmful subcutaneously, would not then cause a true state of shock. The fact is not impossible "a priori."

During the past years, various foreign authors [17], who were surprised by the constant and massive contamination of the wounds in shock patients, have marked their tendency to make veritable shock agents of the substances liberated by the infecting microbes. Above all, they incriminated the perfringens bacillus, its various components, and also the cellular disintegration products caused by this bacterium in the injured tissue. We think that new research is necessary to firmly establish this viewpoint. But, in any case, the toxic substances in question, for the same reason as the endotoxins, should be considered only as triggering factors, their action being most likely limited to starting the general mechanism of shock.

c) But this mechanism consists of what? This great question remains in its entirety.

We recalled above the several mechanisms already invoked concerning the method of action of the endotoxins. It is significant to remark that it is these same mechanisms which have been advanced to describe the onset of traumatic shock. Depending upon their particular tendencies, the authors here again thought either of nervous reactions or of glandular reactions (the interest being concentrated chiefly on the suprarenal hormones). All of these authors have presented serious arguments; so it is probable that in the production of a state of shock, nervous and hormonal causes are intertwined.

Irrespective of the nature of these causes (still obscure, we must recognize), one fact at least appears certain. A traumatic shock is always accompanied by extremely grave disturbances of the circulatory system [18]. But, as we have shown, in the toxic states produced by the endotoxins, it is also the circulation which is the seat of the deepest troubles. Under these conditions, the entire problem of the pathogeny of shock, as well as the entire problem of the method of action of the endotoxins, consists of finding the cause of these disturbances. To equate, as we have done, traumatic shock and intoxication by the endotoxins, should facilitate their solution.

In fact, in studying the method of action of the endotoxins, we can henceforth use the very numerous and valuable documents already produced by research on the mechanism of shock. Thus, the march of progress risks being guided and activated.

From another standpoint, it appears certain that the study of the method of action of the endotoxins could be used to elucidate that which remains obscure in the syndrome of shock. We need only the following example for proof:

We know that wounded patients in shock are particularly liable to infections, and serious infections. To date, we did not know how to explain this predisposition. At best, we could blame the anoxemia which is generally present in the traumatized tissues and which favors the pullulation of certain bacteria, particularly the anaerobics (parfringers). Today, we know the true explanation. In fact, we have shown that the increased sensitivity of the patients to the bacterial aggressions results from the suppression of diaporesis in their organism [19]. But if we were able to find the solution of this problem, it is because our previous research on the method of action of the endotoxins already indicated it clearly [20].

## CONCLUSIONS

I. The state of intoxication produced in the sensitive animal by a bacterial endotoxin is accompanied by deep circulatory troubles.

II. These are the same troubles that we find in subjects in traumatic shock.

III. We can, therefore, equate these two states and henceforth classify the endotoxins among the toxic factors capable of causing shock. The authors show the possibilities arising from this comparison.

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